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**Disease potential of feline leukemia virus (FeLV) collected from
Iberian lynxes (*Lynx pardinus*): low pathogenicity in experimentally
infected specified pathogen-free domestic cats**

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1. Summary

The Iberian lynx (*Lynx pardinus*) is considered the most endangered felid species in the world, less than 250 animals left. The narrow genetic basis may contribute to render this species particularly susceptible to infectious diseases. Within a six-month period starting in December 2006, a feline leukemia virus (FeLV) outbreak of surprising virulence killed about 60% of infected animals. Sequencing of the FeLV envelope surface unit gene revealed a common origin in all lynxes. The sequences were closely related to FeLV-A/61E, originally isolated from a cat in Colorado (1, 2) and only mildly pathogenic in domestic cats. Nevertheless, a particular virulence of the FeLV strain found in Iberian lynxes could not be ruled out. In order to evaluate the virulence of the lynx FeLV, we assessed the disease-inducing potential of the Iberian lynx's FeLV strain in its probable original host, the domestic cat. Intraperitoneal inoculation of specified pathogen-free (SPF) domestic cats with FeLV-infected Iberian lynx blood did not lead to a particularly severe outcome. Thus, the FeLV epidemic in the Iberian lynxes seems to be more related to a potential primary immunodeficiency of these animals than to an extremely virulent FeLV strain.

2. Introduction

The Iberian lynx (*Lynx pardinus*) is the most endangered felid in the world. Not more than 200-250 animals remain, confined in two isolated populations in southern Spain, in the Doñana National Park and Sierra Morena, respectively (3, 4). The genetic diversity in the Iberian lynxes is very low, especially in the Doñana population (5), and may contribute to render this endangered species particularly susceptible to pathogens and possibly even to opportunistic infectious agents. In late 2006, an outbreak of feline leukemia virus (FeLV) killed 6 out of 10 viremic animals within a six-month period (6). FeLV is a gammaretrovirus naturally occurring worldwide in domestic cats and some

small felids (7, 8). Four subtypes of FeLV are known, FeLV-A, -B, -C and -T (9-13). While FeLV-A is present in all FeLV-infected cats, FeLV-B arises by recombination between exogenous FeLV-A and endogenous FeLV-related sequences. FeLV-C and FeLV-T occur through mutation of FeLV-A and can cause pure red cell aplasia or fatal immunodeficiency syndrome, respectively. Infection usually occurs through the oronasal route with an initial replication in lymphoid tissues of the oropharynx, followed by replication in rapidly dividing cells across the body. Once the bone marrow is infected, the virus replicates extensively and viremia develops which in turn affect further organs and tissues of the body (14, 15). Four different outcomes of the infection are known: abortive (no virus detected in blood after exposure, with or without bone marrow infection (16)), regressive, progressive, and the rarely focal or atypical infection (no viremia, virus replication only in defined tissues) (17-22). The majority of cats exposed to FeLV develop regressive infection (with or without viremia) characterized by the elimination of the virus from blood (although proviral DNA remains detectable (19, 23)) and production of neutralizing antibodies through effective immune response (7, 24, 25). Latency, for example in the bone marrow can persist for years (7, 26), while the cats stay healthy but the virus can be occasionally reactivated through immunosuppression (27). Progressively infected cats shed the virus continuously, they can live asymptomatic for up to 2-3 years but usually succumb to FeLV-associated diseases such as for example immunosuppression, degenerative disorders of the hematopoietic system (e.g., anemia) or neoplastic diseases (e.g., lymphomas or leukemia) (28-31). The course of infection is influenced by host and viral factors (e.g., virus subtype), co-infections and/or stress (7, 17, 22, 32-35). Resistance to persistent viremia increases with age (36). There is still no curative therapy for FeLV but effective vaccines are available which protect against persistent viremia and virus spread within a population (37-41).

The dramatic infection outcome in Iberian lynxes was rather unexpected, since in persistently viremic, experimentally FeLV-infected domestic cats the six-months survival rate is of about 90% and the mean survival time of FeLV-positive field domestic cats is about one year (42). In addition, the rare FeLV infections in the Iberian lynxes detected before 2006 did not lead to severe outbreaks (6, 43, 44), probably because of the solitary lifestyle of these felids. Studies in several populations of wildcats from Europe and the Middle East have shown a relatively high prevalence of FeLV antigenemia (8, 45-47), and at least another FeLV outbreak has been characterized in the Florida puma (48). Although, these FeLV infections do not appear to have the same dramatic consequences as the one seen in the Iberian lynxes in 2006/ 2007. Furthermore, the highly pathogenic FeLV-C or FeLV-A variant strains like 61C (which causes together with strain 61E the Feline acquired immune deficiency syndrome (FAIDS) (49)) were not present in the FeLV-positive Iberian lynxes (6). Another atypical hallmark of the FeLV outbreak was the clear association with hemotropic mycoplasmas infection (6), while recent studies in domestic cats failed to detect this relationship (50, 51). So far, it is not clear if this association is linked to host genetics (i.e., a general immune system dysfunction of the Iberian lynxes) or to a peculiar characteristic of the infecting virus strain that may have rendered the host more susceptible to specific pathogens.

To avoid the spread of FeLV, in 2007 progressively infected lynxes were removed from the field, the non-infected animals were vaccinated against FeLV, and the management practices changed in order to reduce intra-specific contact and contact between lynxes and domestic cats (e.g., modification of feeding stations, cat removal) (52, 53). So far, these measures have been successful: since 2007 the only new case had been from a mother-to-child transmission in late 2009 (Guillermo López, personal communication). Nevertheless, the potential pathogenicity of the FeLV strain carried by the Iberian lynxes cannot be neglected: retransmission from Iberian lynxes to domestic cats of a

particularly virulent FeLV strain may have profound consequences, as seen for cross-species transmission in other species (53-59). Thus, to assess the disease-inducing potential of the lynx's FeLV in domestic cats, twelve three-month old specific pathogen-free (SPF) domestic cats were inoculated with either blood from a FeLV-viremic Iberian lynx, or with blood from a FeLV-free Iberian lynx mixed with FeLV-A/Glasgow-1 strain; or with the FeLV-A/Glasgow-1 strain alone. The course of infection followed until five months post-infection (p.i.).

3. Materials and methods

3.1. Animals

Sixteen 8-week-old SPF male, domestic kittens (Liberty Research Inc., Waverly, NY, USA) were included in this study. The cats were divided into four groups of four cats each and housed in separate rooms under barrier conditions. Each cat was clinically examined after receipt from the breeder. Blood and serum samples, oropharyngeal, ocular and rectal swabs were collected to verify their SPF and health status. The blood was analyzed for hematological and clinical-biochemical abnormalities and tested by real-time TaqMan PCR or real-time reverse transcriptase (RT)-PCR for the presence of known feline pathogens (6, 60-68). At the arrival in our facility, all cats were negative for FeLV and feline immunodeficiency virus (FIV) provirus, feline parvo- (FPV), calicivirus (FCV), and coronavirus (FCoV); *Bartonella henselae* and the three feline hemotropic mycoplasmas (*Mycoplasma haemofelis* (Mhf), *Candidatus Mycoplasma haemominutum* (CMhm), *Candidatus Mycoplasma turicensis* (CMt)). Conjunctival swabs tested negative for FCV, feline herpesvirus-1 (FHV₁) and *Chlamydia felis*, the oropharyngeal swabs negative for FCV and FHV₁, and all rectal swabs for FCoV and FPV. EDTA serum was negative for FeLV p27 antigen and for antibodies against FCoV, FCV, FHV₁, FPV, and

FIV (21, 69, 70). In addition, the cats were blood typed (ID- Gel Test Feline A+B Typing; DiaMed AG, Cressier sur Morat, Switzerland).

3.2. Experimental design and infection

All cats were infected intraperitoneally at the age of 12 weeks. All inocula were thawed at 37°C and stored on ice until use. Animals of group 1 were inoculated with 1ml of EDTA blood in 20% Dimethyl sulfoxide (DMSO, Sigma-Aldrich, Buchs, Switzerland) from a viremic two-year old male Iberian lynx from the Los Villares rescue center in Spain (Doñana area, Coto del Rey subpopulation), which survived the outbreak in 2006/2007. The blood was stored at room temperature during shipment from Spain to Switzerland (24hrs) and preserved in 20% DMSO after receipt, packed on ice for two hours, stored at -80°C for two days and finally transferred into liquid nitrogen for long-term storing. The blood was positive for FeLV (p27 60% of the laboratories control, 9.8×10^7 copies virus/ml) and for CMhm (6.9×10^5 copies/ml). Animals of group 2 were inoculated with 1ml EDTA blood in 20% DMSO from a free-ranging female Iberian lynx of the same area as the one used for group 1. The blood was shipped at the same time as group 1 blood, and was positive by PCR for CMhm only (3.7×10^5 copies/ml). In addition, the cats of group 2 were inoculated with 0.2ml FeLV-A/Glasgow-1 strain (500'000 focus-forming units (FFU), p27 195% of the laboratories control, 1.35×10^{10} virus copies/ml) (71). Animals of group 3 were infected with 0.2ml FeLV-A/Glasgow-1 strain (500'000 FFU) in 20% DMSO and 0.6ml RPMI Medium (Invitrogen AG, Basel, Switzerland), and finally animals of group 4 inoculated with 1ml RPMI Medium (Invitrogen AG) in 20% DMSO. Animals of this latter group served as age-matched negative control. Blood samples from the two lynxes were of the same type (A) as the SPF cats. All cats were monitored daily for signs of illness after virus inoculation; body temperature and weight were recorded at each sampling day. To avoid unwanted

effects from cryptic infections from the lynx blood, a thoroughly health check was performed again at week 16p.i. as described above (3.1).

3.3. Sample collection and processing

EDTA blood samples were collected under light anesthesia (3mg/kg S-Ketamin (Keta-S®; Dr. E. Graeub AG, Bern, Switzerland), 5µg/kg Medetomidin (Dorbene®; Dr. E. Graeub AG), and 0.2mg/kg Butorphanol (Morphasol-4®; Dr. E. Graeub AG), administrated intramuscularly) at the time point of infection (week 0), and at week 1-6, 8, 10, 13, 16, and 20p.i.. EDTA-anticoagulated plasma was recovered from blood after centrifugation for 10min at 3'000rpm in a table-top centrifuge. All samples were immediately frozen at -80°C until they were processed.

3.4. Hematology

Hemograms were performed using a Sysmex XT-2000iV (Sysmex Corporation, Kobe, Japan) (72). Packed cell volume (PCV) values between 33% and 45% (5% and 95% quantiles) and leucocytes between 4'600/µl and 12'800/µl (5% and 95% quantiles) were considered to be within the reference range. Diff-Quick-stained (Medion Diagnostics, Duding, Switzerland) smears were evaluated by light microscopy for leukocyte differentiation.

3.5. Detection and quantification of FeLV proviral and plasma viral RNA loads and of *Candidatus Mycoplasma haemominutum* (CMhm) by real-time PCR and RT-PCR

Total nucleic acids (TNA) were extracted from an EDTA-anticoagulated blood volume containing 10⁶ leucocytes, or from a maximum volume of 100µl, using the MagNA Pure LC Total Nucleid Acid Isolation Kit (Roche diagnostics AG, Rotkreuz, Switzerland). Cell copy numbers, FeLV proviral DNA quantities and CMhm loads were determined by real-

time PCR as described (60-62). For detection of FeLV viral RNA in plasma, TNA were extracted from 200µl EDTA plasma using the MagNA Pure LC Total Nucleic Isolation Kit (Roche) and quantified by real-time RT-PCR as described (60).

3.6. Detection of FeLV p27 antigen and FeLV antibodies by ELISA and Western Blot

FeLV viremia was determined by p27 double antibody sandwich ELISA as described (73). Results were calculated as the percentage of a positive control (culture supernatant of FL-74 feline lymphoblastoid cell line permanently infected with FeLV), which was assayed with every plate. Values above 4% were considered to be positive. In agreement with the European Pharmacopoeia (2005), a cat was designated progressively infected when FeLV p27 antigen was positive for three consecutive weeks or on five occasions between weeks 3 and 15p.i.. Anti-FeLV whole virus antibodies were determined by ELISA using 100ng of gradient purified FL-74 FeLV with a dilution of 1:100 as described (74). Values above 25% of a SPF-serum pool were considered to be positive. In addition, FeLV antibodies were detected by Western blot in week 0 and 20p.i. as described, using serum obtained from a pool of immune cats and a mixture of monoclonal antibodies against p27, p15E and gp70 as a positive control (75) (+Ref: siehe Andrea Major Paper Ref. Nr. 26 und 30, ref 28 ist deine #75).

3.7. FeLV subtyping

Two microliters of blood TNA extracts of all cats from week 20p.i. (except Q1 and Q2 which had to be sacrificed for humane reasons at week 10, respectively 13p.i.), and from the FeLV viremic lynx used for the transmission experiment, were analyzed by conventional PCR for presence of FeLV-A, -B and -C using previously described primers (table 1) (76, 77). Each reaction contained a final concentration of 500nM of each primer with 0.4µl 10mM dNTP, 4µl 5x Phusion HF Buffer, 0.2µl Phusion Taq

Polymerase (2U/μl, Finnzymes, Keilaranta, Finland) in a final volume of 20μl, and was amplified on a Biometra gradient cycler (Labrepco, Horsham PA, USA) under following conditions: initial denaturation of 2min at 98°C followed by 40 cycles of 98°C for 20sec, 52°C (FeLV-C) or 64°C (FeLV-A/B) for 30sec and 72°C for 80sec with a final elongation step of 72°C for 10min. PCR products were visualized by agarose gel electrophoresis.

Table 1. FeLV subtyping primers.

Name	5'-3' sequence	Length (bases)	Product length (bases)
FeA-F RB59	CAATGTAAACACGGGGCAC	20	1071
FeA-R RB17	TAGTGATATTGGTTCTCTTCG	21	
FeB-F RB53	ACAACGGGAGCTAGTG	16	857
FeB-R RB17	TAGTGATATTGGTTCTCTTCG	21	
FeC-F RB58	AGATCTTGGGCACGTTATTCC	21	1754
FeC-R RB47	TTGTGAAATGGCATTGCTGC	20	

3.8. FeLV virus titers

Tissue Culture Infectious Dose 50 (TCID₅₀) was calculated using five-fold serial dilutions of the viruses. Six wells of a 24-well-plate with each 10'000 QN10 cells per dilution were incubated with 250μl virus dilute in cell culture medium (78) for 2 hours at 37°C, the inoculum washed away and the cells cultured further in 400μl medium for 8 days with addition of 400μl medium after 4 days. Presence of infectious virus was determined by p27 ELISA as described (74), and TCID₅₀ was calculated using the Karber formula (79).

3.9. Statistics

Statistical analyses were conducted using GraphPad software version 5.00 (GraphPad Software Inc. La Jolla, CA, USA). Longitudinal effects on FeLV viral, FeLV proviral and CMhm loads of the different groups were compared to each other using two-way

repeated measures ANOVA with Bonferroni post-test. Outcomes between groups were compared using Fisher's exact test. P-values < 0.05 were considered to be statistically significant.

4. Results

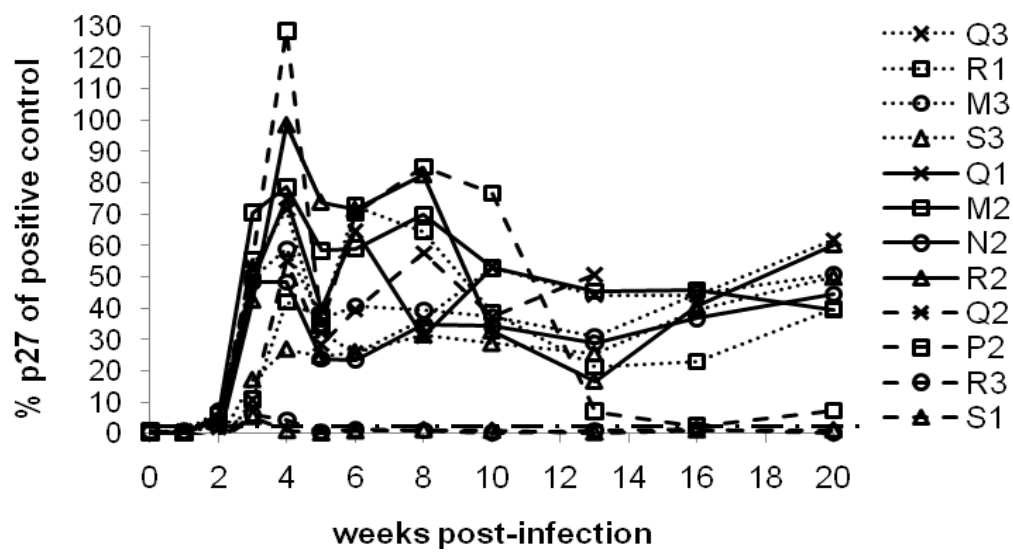
4.1. Pathogen detection in Iberian lynx blood

The obtained lynx blood was PCR/RT-PCR negative for all tested feline pathogens except FeLV (p27 60%, provirus 9×10^6 copies/ml blood, viral RNA 9.8×10^7 copies/ml plasma, titer of approximately 2'000 FFU/ml estimated by TCID50 comparison with the Glasgow-1 stock virus used for group 2 and 3 infection), and CMhm (6.9×10^5 copies/ml) - lynx blood used for group 1; or CMhm (3.7×10^5 copies/ml) alone - lynx blood used for group 2. The lynx plasma used to infect cats of group 1 was seropositive for FCV (1:320), whereas no antibodies were found to all other tested pathogens.

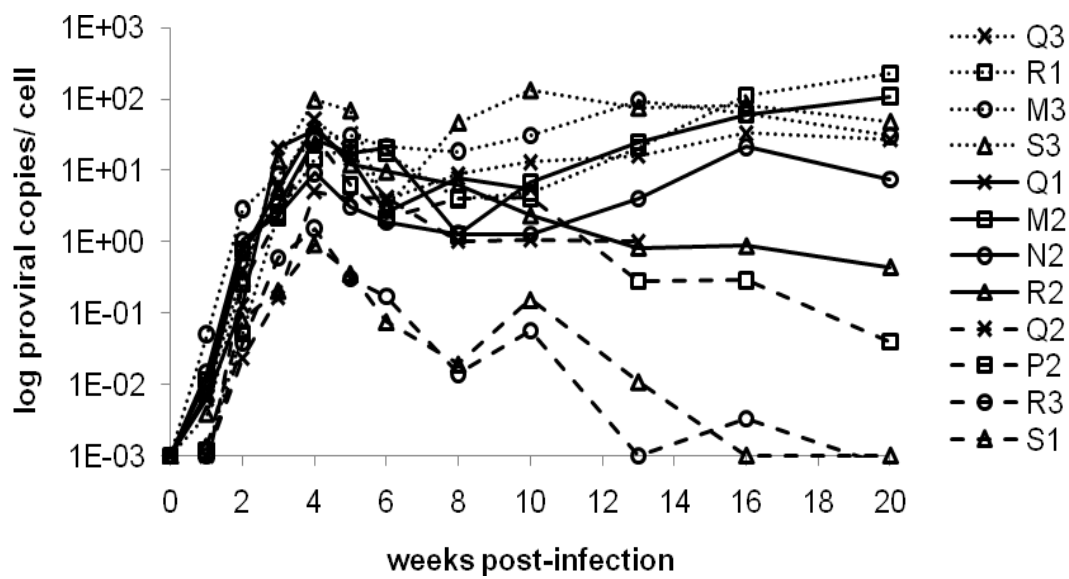
4.2. Outcome of FeLV infection

4.2.1. FeLV DNA, RNA and p27 antigen detection, subtyping

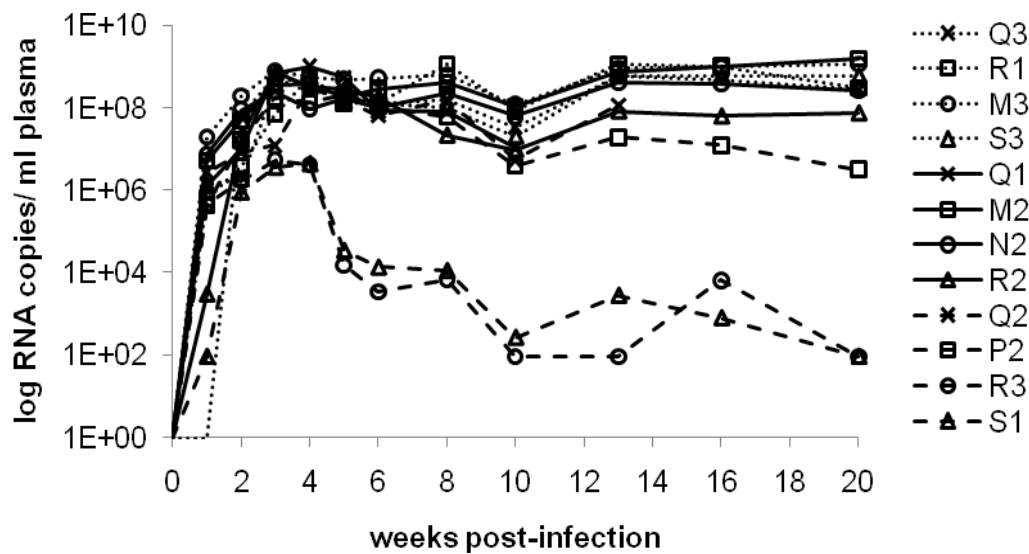
According to the results obtained from p27 ELISA, the four cats of groups 2 and 3, and two of group 1 (P2, Q2) developed a progressive infection. Two cats from group 1 (S1, R3) were regressively infected (figure 1a). All cats infected became FeLV provirus and viral RNA positive, indicating that FeLV infection had occurred in all of them (figure 1b and c). FeLV-B was detected in one cat (R1, group 3) at euthanasia five months post-infection, all others were positive for FeLV-A subtype alone. All cats of group 4 were negative for all tests throughout the experiment.



(a)



(b)



(c)

Fig.1 FeLV antigen p27 (a), proviral (b), and plasma viral RNA (c) loads during the course of experimental FeLV-A infection. The dotted/broken line in (a) represents the threshold for p27 positive results ($\geq 4\%$ of the positive control). Group 1: cats Q2, P2, R3, S1. Group 2: cats Q1, M2, N2, R2. Group 3: cats Q3, R1, M3, S3. Data points for cats Q1 and Q2 are missing starting from week 13 and 16p.i., respectively because the cats had to be sacrificed for humane reasons. There was no statistical difference in the outcomes (regressive vs progressive) between the different groups. FeLV p27, viral and proviral loads were not statistically different between the groups.

4.2.2. Humoral immune response

Seroconversion to FeLV was found in all cats except in those of the negative control group. The cats turned positive starting at week 1-2 after infection. Three group 1 cats (P2, R3, S1) showed a high humoral immune response against FeLV until the end of the experiment, while only two out of seven progressively infected cats (M3, S3) were still positive for FeLV whole virus antibodies (figure 2).

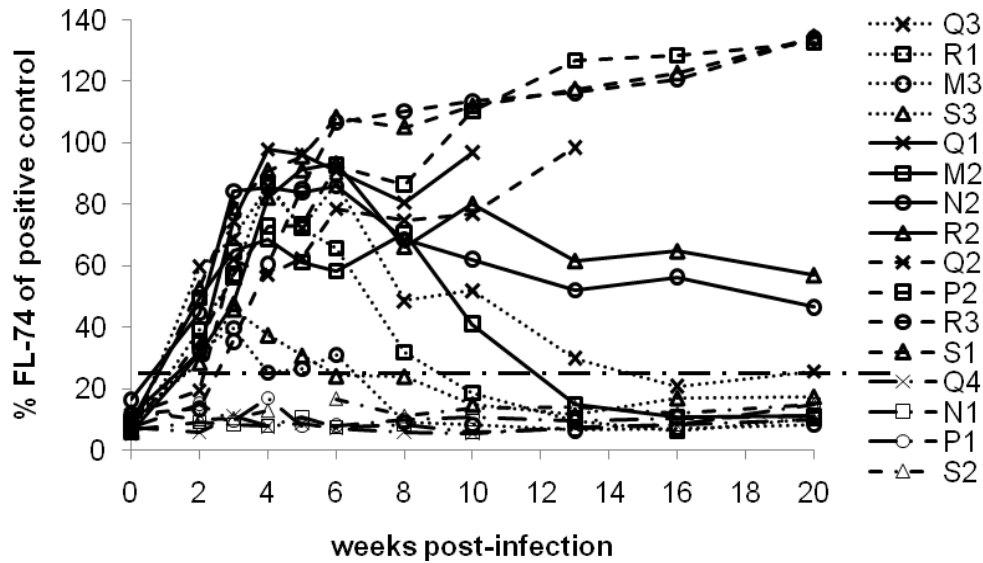


Fig.2 FeLV whole virus (FL-74) antibodies recorded during the study against weeks post infection. The dotted/broken line represents the threshold for positive results ($\geq 25\%$ of the positive control). Group 1: cats Q2, P2, R3, S1. Group 2: cats Q1, M2, N2, R2. Group 3: cats Q3, R1, M3, S3. Group 4: cats Q4, N1, P1, S2. Data points for cats Q1 and Q2 are missing starting from week 13 and 16p.i., respectively because the cats had to be sacrificed for humane reasons.

Antibodies levels to FeLV varied significantly over time between the groups ($p=0.0017$). The difference was caused mainly by group 1 starting from week 8 ($p_{\text{Bonferroni}} < 0.001$ for weeks 8 to 20p.i.). Serum of four infected cats was Western blot positive at week 20p.i. (figure 3). The regressively infected cats R3 and S1 were positive for p58, p27 and p15E, cat P2 weakly for p58 and strongly for p15E. Cat Q1 was weakly positive at week 10p.i. for P15E.

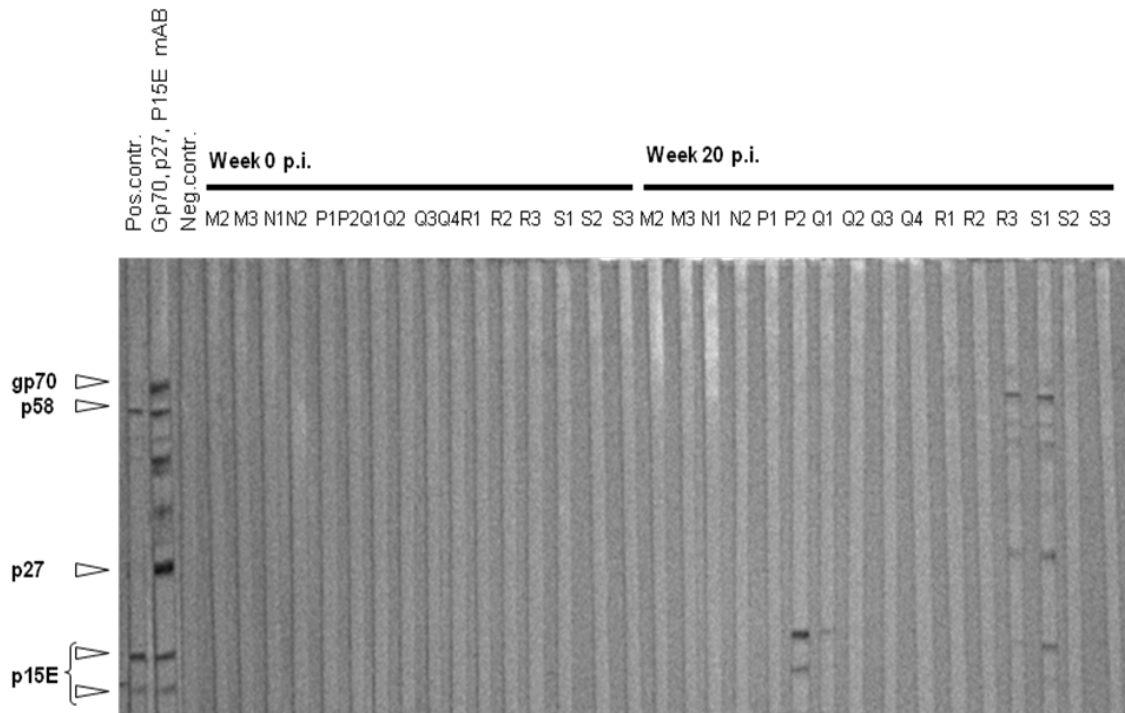
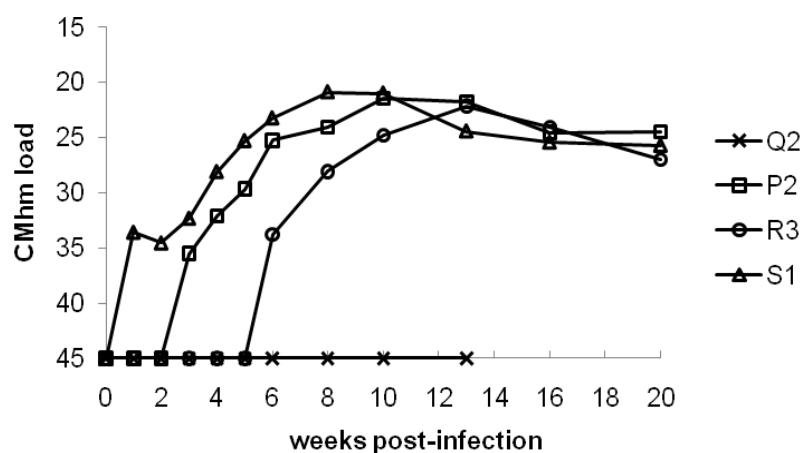


Fig.3 FeLV Western blot analysis of 1:100 diluted plasma samples (diluted 1:100) from week 0 (prior to challenge) and week 20p.i. of the 16 experimentally infected SPF cats. Data for cats Q1 and Q2 are of week 10 and 13 p.i., respectively because the cats had to be sacrificed for humane reasons. The positions of FeLV p15E (two bands), p27, p58 and gp70 are highlighted (open arrows). Positive controls: serum obtained from a pool of immune cats (pos. contr.) and mixture of monoclonal antibodies against FeLV gp70, p27, p15E and respectively (Gp70,p27,p15E mAB).

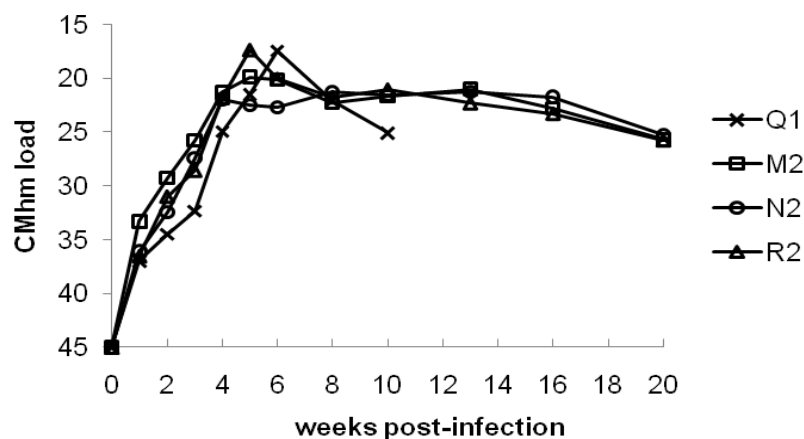
4.3. Outcome of CMhm infection

All cats were hemoplasma PCR negative at week 0p.i.. While all four cats of group 2 became positive by week 1p.i., three cats of group 1 were positive starting at week 1, 3 and 6p.i. (figure 4a and b). One cat (Q2) never became positive in blood during the whole experiment. No significant difference in copy numbers was found after week 6p.i. between the groups. However, the overall course of CMhm infection was significantly different between the two groups ($p=0.0319$), especially in weeks 4 and 5 ($p_{\text{Bonferroni}} <$

0.01). Clinical signs of CMhm infection were absent in the recipient cats. Anemia was observed in one cat Q2 (group 1), in which the PCV dropped from 25% to 18%, however this cat was never positive for CMhm in blood.



(a)



(b)

Fig.4 Time course of CMhm loads in experimentally infected SPF cats. Group 1 (a) and group 2 (b) loads are expressed as 45 minus the cycle threshold (Ct) values measured. Data points for cats Q1 and Q2 are missing starting from week 13 and 16p.i., respectively because the cats had to be sacrificed for humane reasons.

4.4. Hematology and clinical disease

All cats and lynxes were blood type A. The cats showed some fluctuation in mean PCV values during infection but values remained within the reference range throughout the study. PCV and white blood cells showed no significant change between groups. Two cats had to be euthanatized during the experiment. Cat Q1 (group 2) developed a fatal panleukopenia at week 10p.i., while cat Q2 (group 1) developed skin lesions on the back, head and neck during week 8 to 10p.i., and later on an edema in the belly. At week 13p.i. the PCV declined from 25% to 18%, with a low total protein of 32.8g/l, and albumin of 11.6g/l, and an increased lipase 558 U/L and amylase of 2'241 U/L. In autopsy, next to the distinct edema, a middle-grade acute pancreatitis and omentitis was found.

4.5. Post-infection health test

At week 16p.i. a second health test was performed to rule out the influence of cryptic infections in the lynx blood. Indeed, cat P2 (group 1) was found to be seropositive for FCV (titer 1:160) and FHV₁ (titer of 1:320). Conjunctiva and saliva swabs extracted and tested by RT-/PCR were negative at this time point. Tests of all earlier time points revealed that this cat became FHV₁ PCR positive only once in blood in week 1p.i. (Ct 36.9), where it was weakly seropositive (titer of 1:20) as well. FHV₁ serology was positive at weeks 10 and 13p.i. (titer of 1:40 and 1:160) as well, FCV serology positive first at week 13p.i. (titer of 1:80).

5. Discussion

The aim of this study was to assess the pathogenic potential of the FeLV strain circulating among Iberian lynxes in 2006/2007 outbreak in case of possible retransmission to resident domestic cats. We therefore obtained EDTA-blood from a viremic Iberian lynx that was infected in 2006 and held in the Los Villares Rescue Center in Spain since then. Because no FeLV-infected Iberian lynx is free of co-infection with hemotropic mycoplasmas (6), FeLV-free Iberian lynx blood, which was also co-infected with the same mycoplasma strain (CMhm), had to be obtained as matched control. In addition to the determination of cross-transmission potential of FeLV from Iberian lynxes to domestic cats, this is, to our knowledge, the first report of successful hemotropic mycoplasmas infection transmission after 24h storage of the blood at room temperature followed by cryopreservation. To rule out possible influences of CMhm co-infection in the pathogenesis of FeLV infection, SPF domestic cats were infected either with the FeLV-A/Glasgow-1 strain alone (group 3) or with FeLV-A/Glasgow-1 and the CMhm-positive blood (group 2). The course of infection in these two groups was identical. All developed a progressive FeLV infection and in only one cat (group 3) the more pathogenic FeLV-B strain was observed, while all cats were negative for FeLV-C. The course of CMhm infection was identical in all cats of group 2, with a bacteremia peak during weeks 4 to 5. One cat from group 2 (Q1) developed a fatal panleukopenia, at week 10p.i. which may have been caused through FeLV replication in the bone marrow. FeLV-induced panleukopenia has been recognized for many years (7, 22). Thus, an eventual contribution to increased pathogenicity of FeLV from intrinsic factors present in the Iberian lynx blood can be excluded.

Two of the cats (S1, R3) from group 1 (infected with FeLV-positive Iberian lynx blood) were regressively infected and two (Q2, P2) progressively infected according to p27 ELISA outcomes. Because of the small group size, the outcome was not statistically

different from the other two groups. Nevertheless, whole virus antibodies levels were significantly different towards the end of the experiment and Western blot analyses suggested that a third cat from group 1 (P2) may have become regressively infected as well.

This outcome can be seen as in accordance with the low titer of the virus used for infection (about 1'000 times lower than the Glasgow-1 stock), which may have hampered the establishment of an effective progressive infection. However, in group 1 no difference from known FeLV infection outcomes was observed. Although the cause of disease in cat Q2 is still unclear, biopsy of a skin lesion (neck) was positive for FeLV sequences and FeLV antigens as demonstrated by RT-PCR and immunohistology, respectively (data not shown). This may explain the sudden lesions, as ulcerative dermatitis is described for FeLV-infection in some cases (80). A possible protein loss over the intestines may have been responsible for very low protein concentration loss and development of edema, a pattern associated with FeLV-related diseases such as an intestinal lymphoma. Interestingly, although intestines, liver and kidneys were carefully examined for presence of lymphoma, no evidence of neoplasia was found. Thus, the etiology of the extremely low plasma protein concentration was not elucidated.

Cat P2 showed signs of contact with FHV₁ (PCR positive in week 1p.i. and seropositive afterwards) and FCV antigen (seropositivity), revealing that these two pathogens had been present in the lynx blood in quantities below the detection limit. Nevertheless, these subclinical infections, introduced through lynx blood inoculation, did not influence the outcome of FeLV infection, since this cat was progressing towards a regressive infection. The course of CMhm infection in group 1 was significantly different from group 2, despite of the nearly identical loads in the blood utilized for infection. A possible explanation may be a difference in CMhm viability: while real-time PCR assays provides

reliable quantification of CMhm in blood samples (62) the true correlation between copy number and the number of living organisms would require quantitative culture which is not yet possible (81). A higher infectivity of the inocula and/ or intraperitoneal (obligatory because of the experimental infection with FeLV) rather than intravenous administration may explain the differences with already described courses of CMhm infection, where the loads peaked in weeks 2 to 4 (82, 83). Alternatively, the different infection course of CMhm may have been influenced by FeLV infection outcome itself, but this was not testable in the experimental setting that was used. Although a relationship between retroviruses (e.g., FeLV or FIV) and hemotropic mycoplasmas co-infection has been postulated by different authors (35, 83-85), recent field studies did not find any association (50, 51). The results presented support the latter findings and show that a retrovirus-positive status with CMhm co-infection will not become manifest in a particular progression or outcome of disease on a short term.

The low pathogenicity of the transmitted FeLV strain in SPF cats underscores the importance of alterations in host-related factors that together with environmental changes may all interact to affect the susceptibility of Iberian lynxes to disease (53). For example, the lack of endogenous FeLV-related sequences (6) might also contribute to a higher pathogenicity of FeLV infection in the Iberian lynxes, since a protective role of endogenously expressed retroviruses has been postulated e.g., for gammaretroviruses in mice (86-89). The lack of exposure to pathogens (e.g. FeLV, FIV, canine distemper virus (CDV)) might have made the Iberian lynx vulnerable to outbreaks of these diseases (44). Nevertheless, the most important factor may be the loss of genetic diversity as a consequence of inbreeding (especially in Doñana region), which ended up in the apparent lack of acquired immunity and immunocompetence of the Iberian lynxes against infectious agents (5, 47, 90). A primary immune deficiency of the lynxes may have made this species more vulnerable to infectious diseases, a fact which should be

thoroughly investigated and which may pose a real threat to the future survival of this species.

6. Conclusions

In conclusion, the present study shows that the FeLV virus responsible for the outbreak in Iberian lynxes in 2006/ 2007 is apparently not extremely pathogenic when inoculated into SPF domestic cats. Hence, we assume that there is no increased risk in case of lynx-to-cat retransmission.

7. References

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